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ENTERIC VIRUSES CIRCULATION IN THE ENVIRONMENT AND THEIR OCCURRENCE IN CASES OF INFANTILE GASTROENTERITIS

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Introduction: Infective gastroenteritis (GA) represent a world public health relevant problem. Enteric viruses are the pathogens mainly involved in the episodes of GE, causing about 70% of the cases. In Italy most of the GA are not notified because of a mild symptomatology which does not require medical support. Only in cases of hospitalization (prevalently children) the cause of the disease is further investigated. However the viruses usually searched are the pathogens more commonly associated to GE (rotavirus, norovirus, and adenovirus) and very often (in 1/3 of the cases) the patient is discharged without knowing the infective agent. The diagnostic gap lead us to hypothesize that there are other viruses involved, as demonstrated by recent surveillance studies which show interesting prevalences of emerging viruses. In the present study we investigated the presence of 10 different viruses in the faeces of children hospitalized with GE in the Campania Region (Southern Italy). The same viruses were also investigated in samples of marine water and shellfish from the coastal area surrounding the Region with the aim to trace a picture of their circulation in the environment.
Materials and methods: We analyzed 70 faecal samples taken from children (5 months-10 years old) hospitalized with gastroenteric sympthoms. We furthermore investigated 155 sea water samples taken from discharge points situated along the coast of the Region and 34 mussels samples (made of around 30 animals each) farmed in the Gulf of Naples. Waters were preliminary concentrated from 10 liters to 50 ml by filtration; faeces (100 mg) were first suspended in 900 µl phosfate saline buffer; epatopancreas of 10-15 mussels (per sample) were pooled together and underwent a preliminary virus extraction step. Nucleic acids extraction was carried by KingFisher Flex authomatic extraction system using the MagMax viral/pathogen II nucleic acid isolation kit. Each extract underwent Real time PCR analyses for the detection of the following viruses: adenovirus (AdV), norovirus GI and GII (NGI, NGII) astrovirus (AsV), sapovirus (SaV), aichivirus (AiV), rotavirus (RV), parechovirus (ParV), salivirus (SalV) and enterovirus (EV). For each viral target a protocol with specific primers and probes was carried out. NoV and AdV positive samples were further characterized by sequencing.
Results: Results showed that 58.6% of the feces (41/70) were positive to at least one virus. The virus most frequently identified was AdV, present in 24/41 samples (58.5%), followed by NGII (22/41, 53.7%), RV (11/41, 26.8%) and ParV (8/41, 19.5%); SaV, AiV and SalV were found only in 1-2 samples, while the other viruses (NGI, AsV and EV) were never identified. Looking at seawaters, 45/155 (29%) were positive to at least one virus with RV the preva-

lent one (22/45, 48.9%), followed by AiV (14/45, 31.1%), NGI and SalV (both 7/45, 15.5%), ParV (5/45, 11.1%) and NGII (3/45, 6.6%). EV, SaV and AdV were found only in few samples (1-2) while AsV was always negative. Mussels were 38.23% (13/34) positive to at least one virus. Results revealed the predominant presence of the two NoVs: GI 84.6% (11/13 samples), and GII 23.1% (3/13). SalV and RV were the only other two viruses identified (in 1-2 samples). Characterization of NGII discovered the occurrence of various genotypes: GII.33, GII.2, GII.1 (GII.P33), GII.17, with GII.2 the most frequent one. As to AdV we identified the serotypes 41 and B3. Of the NGI positive samples only one mussel gave a good sequence and the strain was genotyped as GI.3.

Discussions: Environmental samples showed an intense circulation of the enteric viruses, with the presence of all the investigated ones (but AsV). More than 50% of the feces exhibited at least one virus, some of them (17) showing the co-presence of more than one pathogen, with NGII and AdV almost always found together. One sample, belonging to a 8 months years old baby, was even contaminated by 4 viruses (NGII, SaV, AdV and ParV). The pathogens more prevalent in the feces where AdV and NGII, (both revealed in more than 50% of the samples). RV showed a definitely lower prevalence (around 1/4 of the samples) even if it was the main circulating virus in the environment. Considering that RV is the most common cause of GE in children < 5 years in the world, with a full range of severity of clinical presentations often requiring hospitalization, we could conclude that its lower involvement in GE (also with respect to high viral circulation) could be attributed to implementation of the vaccination plan in our Region. Surprisingly we identified ParV, an emerging virus, in 1/5 of the samples: further investigations would clarify its involvement (if any) in the GE episodes.